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Increased Susceptibility of Bobwhites (Colinus virginianus) to Histomonas meleagridis after Exposure to Sevin Insecticide[A]

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SUMMARY

Bobwhites given heterakid eggs but no Sevin became infected with cecal histomonads, but there was no pathological histomoniasis. Quail given 50 μg of Sevin (10 μg/day) behaved normally, but at necropsy they had slightly discolored livers. Quail given various doses of heterakid eggs and Sevin (Sevin increasing from 2.5 to 50 μg) and those given various doses of heterakid eggs and 10 μg/day of Sevin developed pathological histomoniasis and mortality rates of 36 and 63%, respectively.

INTRODUCTION

Bobwhite quail resist infection by or are asymptomatic carriers of the protozoan parasite *Histomonas meleagridis* (3,9,11, 13,17). The chicken cecal worm *Heterakis gallinarum*, in nature, can parasitize bobwhites (4,8,12,13,14), but bobwhites are marginal hosts (13,14). Until recently, whether *Histomonas meleagridis* can infect bobwhites was uncertain (3). The fact that other diseases, such as candidiosis, leukosis, cholera, salmonellosis, tuberculosis, or combinations of some diseases, could mimic histomoniasis made clinical diagnosis difficult. In only a few cases was the disease positively diagnosed in the laboratory by culturing the organism (3,9,17).

Sevin (1-napthyl *N*-methyl carbamate, Union Carbide, New York) is a broad-spectrum insecticide that inactivates cholin-

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Table 1. Effects of *Heterakis gallinarum*, *Histomonas meleagridis*, and Sevin individually and in combination on Bobwhites.

Group	No. of birds	Total dosage of heterakid eggs[A]	Total Sevin (µg single dose)	Cecal histomonads No. of birds	Cecal histomonads Group incidence (%)	Liver lesions No. of birds	Liver lesions Group incidence (%)	Cecal damage No. of birds	Cecal damage Group incidence (%)	Death post-treatment No. of birds[B]	Death post-treatment Group incidence (%)	Death post-treatment Time to death (days post-treatment)
1	10	0	0	0	0	0	0	0	0	0	0	0
2	6	1000	0	6	100	0	0	0	0	0	0	0
3	6	0	50[C]	0	0	0	0	0	0	0	0	0
4	2	100	2.5	2	90	0	59	0	71	0	36	0
	2	500	5.0	1		0		0		0		0
	2	1000	10	1		1		1		0		0
	2	1500	15	2		2		2		0		0
	2	2000	20	2		2		1		2		19:25
	2	2500	25	2		2		2		0		0
	2	3000	30	2		2		2		1		19
	2	3500	35	2		1		2		1		19
	2	4000	40	2		1		2		1		20
	2	4500	45	2		1		2		1		20
	2	5000	50	2		2		2		2		19:23
5	2	100	10	2	95	2	71[D]	2	81[D]	1	63[D]	23
	2	500	10	1		0		0		0		0
	2	1000	10	2		2		2		1		19
	2	1500	10	2		1		2		1		19
	2	2000	10	2		2		2		2		23:23
	2	2500	10	2		2		2		2		21:21
	2	3000	10	2		2		2		2		19:19
	2	3500	10	2		0		0		0		0
	2	4000	10	2		2		2		2		19:19
	2	4500	10	2		2		2		2		20:19
	2	5000	10	2		2		2		2		19:19

[A] All eggs were administered in equal increments each day for five days.

[B] P = 0.05 < 0.10, chi-square value at P = 0.05 is 3.84, with the tabular value for total dying equal to 3.36 for Groups 4 vs. 5.

[C] Administered in dosage of 10 µg/day for five days.

[D] Significantly higher than for Group 4.

esterase at synaptic clefts and myoneural junctions. It is a reversible cholinesterase inhibitor that in vertebrates is destroyed or detoxified by liver microsomes (10). It is not stored in tissues and is rapidly metabolized with degraded metabolic products excreted via the excretory system. Orally administered Sevin may be initially detoxified by mucosal cells of the gastrointestinal tract (16).

The question of whether sublethal dosages of a pesticide would increase an animal's susceptibility to a parasite or enhance the pathological effects of the parasite upon the host was investigated. Specifically, we examined whether sublethal dosages of the insecticide Sevin, at levels that might be encountered in nature because of crop dusting, treatment of forage, or contamination of drinking water, would increase the susceptibility of bobwhites to *Histomonas meleagridis*. Sevin was selected because it is commonly applied and is considered relatively safe (7). The dosages were based on concentrations that can be encountered in nature (7).

MATERIALS AND METHODS

Equal numbers of young adult male and female quail weighing from 121 to 285 grams (average weight, 200 g) were obtained from a private game farm at Hutchinson, Kansas. They were vaccinated intranasally with Newcastle vaccine (B1 type, B1 strain), kept in 5-tier floor cages, and fed a commercial ration. Ten birds were selected at random and necropsied to determine that they were not parasitized with heterakids or histomonads.

Heterakid eggs were embryonated and given *per os* to quail by established methods (5,6). The stock insecticide was a wettable powder 1:1 (w/w) Sevin: acidified Pike's Peak Clay. Fifty milligrams of active insecticide suspended in 500 ml of distilled deionized water gave a concentration of 100 μg of insecticide per 1 ml of water. Desired dosages of Sevin were administered *per os* through a calibrated pipette.

Group 1. Ten birds given neither heterakid eggs nor insecticide were kept as controls to monitor possible influences of laboratory conditions.

Group 2. Each of six birds was given a total of 1,000 ($\pm$100) embryonated heterakid eggs, at 200 ($\pm$20) eggs/day over five days (Table 1). That dosage is ample for transovarian transmission of *Histomonas meleagridis* (9,11,12). The repeated administration of several small dosages of *Heterakis gallinarum* eggs provided the best conditions for obtaining burdens of larvae or adults (2,19).

Dosages of heterakid eggs beyond 1,000 may inhibit development of adult heterakids resulting in low worm burdens (1).

All birds were individually examined at death, or when the experiment terminated, for heterakids, histomonads, damaged ceca, and liver lesions.

Group 3. Six quail were given Sevin at 10 μg/day for five days before being killed 25 days post-treatment. Their livers and ceca were examined for pathological effects.

Group 4. Twenty-two quail were given 100 to 5,000 embryonated eggs of *H. gallinarum* at dosages shown in Table 1. Initial egg administration began one day before administration of the insecticide and continued in equal increments (daily for 5 days) until all eggs were given. The insecticide was given as a single dose according to the schedule in Table 1. At 25 days post-treatment (or at death), liver and cecal contents were examined for histomonads and heterakids.

Positive confirmation of the presence of the protozoan *Histomonas meleagridis* for all experimental groups was by microscopic examination of fresh cecal and liver contents from suspect birds. Further confirmation was by anal inoculation of turkeys using liver and cecal materials from infected birds employing established techniques (11).

Group 5. Twenty-two quail were treated like those in Group 4, except that Group 5 birds received 10 μg of Sevin as a single dose before being given dosages of from 100 to 5,000 heterakid eggs. The nematode eggs were administered in equal increments within a five-day period (Table 1). The quail were necropsied upon terminating the experiment or on earlier death.

The data were analyzed using chi-square (Yates correction factor).

RESULTS

Group 1. The quail remained in good condition throughout the study. There was no extraneous laboratory infection by heterakids or histomonads (Table 1).

Group 2. All quail had cecal histomonads, but none had pathological evidence of histomoniasis (Table 1), demonstrating that histomonads in heterakids from chickens can be transmitted to quail.

Group 3. Discolored livers resulting from 50 μg of Sevin given at 10 μg/day for five days (Table 1) indicated only slight damage.

Group 4. Sevin dosages of more than 10 μg as a single dose helped induce cecal histomonads and damaged livers and ceca. Deaths from histomoniasis occurred after dosages of 20 μg or more (Table 1).

Group 5. Dosages of 10 μg of Sevin and 100 or 500 heterakid eggs produced inconsistent pathological evidence of histomoniasis (Table 1). Doses of 1,000 or more heterakid eggs along with 10 ppm of Sevin produced pathological histomoniasis. Numbers of quail dying in this group and those dying in Group 4 differed significantly ($P < 0.05$). Times of deaths post-treatment did not differ significantly between Groups 4 and 5. Except for cecal histomonads, incidence of pathological factors was significantly higher in Group 5 than in Group 4 (Table 1).

DISCUSSION

The present study showed that low dosages of Sevin may increase the susceptibility of bobwhites to *Histomonas meleagridis* by allowing them to become susceptible to strains of *H. meleagridis* to which they are normally refractive. Our quail tolerated 50 μg of Sevin, but as little as 10 μg in the presence of cecal histomonads caused fulminant histomoniasis and death.

The Environmental Protection Agency has established 100 ppm (equivalent to our dosage of 100 μg) as the maximum residual tolerance level of Sevin on forage crops (day of application). Depending upon environmental conditions, its half-life is 3–7 days, so the insecticide can exist in nature at dosages we used.

One possible explanation for the results we obtained may be that *H. meleagridis,* normally nonpathogenic in quail, may become pathogenic as a result of damage to cecal mucosal cells during detoxification of the insecticide (16). This could allow the protozoan entrance to an environment from which it is normally excluded by the cecal mucosa. The parasites rapidly invade the liver, destroying the organ or decreasing its ability to detoxify the insecticide.

The characteristic cecal lesions of histomoniasis are caused by the physical and enzymatic activities of the parasite during and after penetration through the cecal mucosa. This penetration results in hemorrhage, leukocytic infiltration, and necrosis of the cecal lining at foci of infection. The resulting lesions are characterized morphologically as inflamed, ulcerated areas that enlarge and anastomose as the disease progresses. As the necrotic tissue is

sloughed into the cecal lumen, it forms a hard, dry, whitish, cheesy material, precluding its elimination from the cecum. The accumulation of this material and thickening of the cecal walls causes gross enlargement, inhibiting cecal function (18).

About the 6th day postinfection, small, whitish, pinpoint spots appear on the liver. These spots are produced when the histomonads invade the liver via the blood supply from the cecum. Liver lesions are disc-shaped and have depressed white to reddish centers. As the disease progresses, these lesions may anastomose to cover most of the liver surface. Similar lesions are occasionally produced in the heart, lungs, kidneys, spleen, and pancreas.

Normally the bobwhite is unimportant in contaminating poultry ranges or yards with *Heterakis gallinarum* or *Histomonas meleagridis*, but they may become infected with these parasites when close to poultry yards (4,13). *Heterakis gallinarum* has been reported in bobwhites in the wild (4,12,15,17), but they are only conditional hosts for this nematode: histomoniasis provides an unfavorable environment for heterakids (13). The use of a chicken strain of heterakids not well adapted to quail to transmit histomonads may explain why no adult *H. gallinarum* were recovered.

The pretreatment of *Histomonas meleagridis* with Sevin before infecting quail with histomonads was not undertaken. The parasite system we used does not readily or directly lend itself to this type of an approach, since *H. meleagridis* is transovarially transmitted to hosts via the eggs of *Heterakis gallinarum*. It is not known which nematode eggs contain the protozoan, nor has it been possible to routinely isolate them from the eggs. Thus, determining what effect the insecticide was having upon the protozoan was not possible. At best, pretreating histomonads in eggs with Sevin would be indirect. Since nematode eggs are somewhat resistant to environmental factors (heat, dessication), the insecticide would probably not penetrate the egg shell at the concentrations used. Future work should be done on the ability of Sevin to penetrate the egg shell in order to determine whether Sevin is directly affecting the pathogenicity of *Histomonas meleagridis* rather than causing some damage to the host.

The simultaneous variation of two variables (heterakid eggs and Sevin dosages, Group 4 only) as one approach to a portion of this problem was necessitated because of the inability to obtain large numbers of adult bobwhites for experimentation. This phase of the experiment attempted to correlate heterakid egg dosages

with Sevin dosages to determine whether a critical combined egg dosage–Sevin dosage existed for inducing pathognomonic histomoniasis.

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